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METABOLIC FLEXIBILITY AMONG WOMEN AFTER A SINGLE HIGH
FAT MEAL

A Thesis
Presented to
The Faculty of School of Kinesiology, Recreation, and Sport
Western Kentucky University
Bowling Green, Kentucky

In Partial Fulfillment
Of the Requirements for the Degree
Masters of Science

By
Alyssa Olenick

May 2017

METABOLIC FLEXIBILITY AMONG WOMEN AFTER A SINGLE HIGH
FAT MEAL

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I dedicated this thesis to my parents, Mary Ann and Randy, who have wholeheartedly supported me in everything I peruse. Also, Dr. Jill Maples, without you I would not have grown into half the scientist I have during this time, and this project would have never been what it is. Lastly, the entire Kinesiology department, each of you has helped shaped me as a student, challenged me, and most importantly, fed and/or caffeinated me during this time, and for that I am forever grateful.

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METABOLIC FLEXIBILITY AMONG WOMEN AFTER A SINGLE HIGH FAT MEAL

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Directed by: Jill Maples, Rachel Tinius, and Lee Winchester

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PURPOSE: Obese women have increased rates of metabolic diseases compared to those of healthy weight status. Additionally, African-American (AA) women have higher rates of metabolic disease compared to Caucasian (CA) women. Metabolic inflexibility is the inability to adjust substrate oxidation in response to dietary intake; potentially leading to weight gain and the development of metabolic disease. Few studies have investigated the impact of weight status and/or ethnicity on the metabolic response of women to a single high fat meal. An acute unfavorable metabolic response may contribute to the higher incidence of metabolic disease among not only obese, but also AA women. Therefore, the purpose of this study was to determine the impact that weight status (lean vs. overweight/obese) and/or ethnicity (CA vs. AA) has on metabolic health in women in response to a single high fat meal. **METHODS:** CA (n= 15; age=26.27±5.65 yrs; BMI=30.72±11.92kg/m²) and AA (n= 12; age=26.75±6.65yrs; BMI=28.32±6.91kg/m²) women consumed a high fat shake (1062 calories, 56% fat). Blood was drawn and resting energy expenditure (REE) and substrate oxidation (estimated using indirect calorimetry) were assessed at baseline/fasted (T1), 120 minutes post-shake, (T2) 240 minutes post-shake (T3). **RESULTS:** Lipid and carbohydrate oxidation significantly increased among all women in response to the high fat meal ($p<0.01$). Significant increases in fat

oxidation were seen from T1-T2 for all women (CA lean: $+57.9 \pm 24.5\%$; CA overweight/obese: $+30.2 \pm 11.8\%$; AA lean: $+10.2 \pm 18.1\%$; AA overweight/obese: $+40.6 \pm 52.6\%$; $p < 0.01$). Among the CA women only, CA lean women displayed a significantly higher increase in fat oxidation in response to the meal compared to CA overweight/obese women, but there were no differences among lean and overweight/obese AA women. Similarly, weight status influenced changes in apolipoproteins after consuming the high fat meal among CA women, but not AA women. CONCLUSIONS: CA lean women displayed the most metabolic flexibility in response to the high fat meal. A metabolic system that is less able to respond to metabolic stimuli such as a high fat diet (as noted in all groups compared to lean CA women) may play a role in the increased metabolic disease prevalence among obese and AA women.

Chapter 1. Introduction

Obesity and the subsequent disease states are a major public health concern in America with over 2/3 of the population considered overweight or obese (Clevenger, Kozimor, Paton, & Cooper, 2014; Muoio, 2014). Along with an increase in metabolic (e.g. diabetes) and cardiovascular disease (e.g. myocardial infarctions), there has been an influx in research associated with the health implications of increased weight status. In addition to this, there has been a notable increased in prevalence of metabolic diseases among African American (AA) women when compared to Caucasian American (CA) woman; this increased prevalence has become a national concern (Malayala & Raza, 2016; Ogden, Carroll, Fryar, & Flegal, 2015).

Human metabolism plays an important role in substrate utilization in response to meal consumption. Metabolic flexibility is defined as the ability for one to appropriately shift macronutrient (i.e. carbohydrates, fat, protein) (or substrate) metabolism appropriately (Storlien et al., 2004). Contrastingly, metabolic inflexibility is an individual's inability to respond appropriately. Metabolic inflexibility has been linked to cardiovascular, metabolic, and insulin resistant disease states (Muoio, 2014).

Previous research comparing metabolic differences between lean and obese subjects has shown lower fatty acid oxidation among obese individuals (Heilbronn, Gregersen, Shirkhedkar, Hu, & Campbell, 2007). Additionally, research suggests that obese women, compared to lean women, are metabolically inflexible (Heilbronn et al., 2007).

Metabolic inflexibility, in response to a typical high fat Western diet, may be a mechanism contributing to the higher incidence of metabolic disease and obesity among AA women. Current literature shows the response of a high fat meal after one to four weeks of a controlled diet between CA and AA women, but it lacks the understanding on how women of different ethnicities respond to only a single high fat meal (Berk et al., 2006; Gerhard et al., 2000). . While reduced fat oxidation among AA women has been shown (Berk, Kovera, Boozer, Pi-Sunyer, & Albu, 2006; Stull, Galgani, Johnson, & Cefalu, 2010), the impact that inflammation and gene expression have on metabolic flexibility after a single high fat meal has not been studied. Understanding how women of different ethnicities respond to high fat meals is important in further understanding potential mechanisms behind the prevalence of diseases in obese and AA women.

Due to the relationship of metabolic inflexibility and chronic disease, such as obesity, a greater understanding of metabolic differences in response to a single high fat meal between lean and obese women is warranted. Additionally, it is not clearly defined whether or not metabolic inflexibility among AA women could be a factor leading to the higher prevalence of obesity and other chronic diseases among AA women. Therefore, the purpose of this study is twofold: 1) to assess metabolic differences, in response to a high fat meal, between lean and obese women and 2) to determine the effect of ethnicity on metabolic flexibility in response to a single high fat meal.

Chapter 2. Literature Review

Metabolic Flexibility

The human diet is composed of three primary macronutrients: fatty acids, glucose, and amino acids, with fuel metabolism primarily coming from carbohydrates and fatty acids. Additionally, human metabolism is controlled by major organs such as skeletal muscle and adipose tissue (Storlien et al., 2004). It is important for the body and primary organs, such as skeletal muscle and adipose tissue, to respond in an efficient manner to the diet one consumes. In order to utilize energy efficiently, these organs must respond and store or use the energy source at hand accordingly. The proficiency of an individual's response on a systemic level to a given energy supply is an important indicator of an overall healthy metabolism (Storlien et al., 2004). Adipose tissue plays an important role in these metabolic processes as it buffers the flux of fatty acids during the postprandial period, where obese individuals have been shown to have a downregulation of hormone sensitive lipase and up regulation of lipoprotein lipase (Galgani et al., 2008). Additionally, skeletal muscle is important as it provides over 20% of our total energy expenditure and regulates glucose metabolism and lipid flux (Storlien et al., 2004). The ability of skeletal muscle to oxidize fats efficiently is not independent of insulin sensitivity. The consumption of a single high fat meal, if not metabolized efficiently, can result in intracellular lipid accumulation and insulin resistance (Galgani et al., 2008; Kelley et al., 2005). Physiological responses to a single high fat meal such as intracellular lipid accumulation and insulin resistance are problematic as they are precursors to

many major disease states such as cardiovascular disease and Type II Diabetes (Galgani et al., 2008; Kelley et al., 2005).

Metabolic flexibility is an individual's ability to adapt to utilize and efficient metabolism an available fuel source (Galgani et al., 2008; Storlien et al., 2004). In contrast, metabolic inflexibility is the inability to shift fuel metabolism, in response to consumption of a particular fuel source (i.e. carbohydrates, or fatty acids) (Galgani et al., 2008; Storlien et al., 2004). For example, an individual displaying metabolic inflexibility would exhibit an inability to properly shift to fat metabolism after consuming a high fat meal. The ability to adjust metabolism in response to macronutrient availability is a marker of overall metabolic health, with the lack thereof being a potential biomarker of cardiometabolic disease and associated disease states (Muoio, 2014). The efficient utilization of dietary fat can result in better long-term weight management and health status, while impaired fat metabolism can lead to an increased circulation of free fatty acids and an accumulation of these as well as triglycerides within skeletal muscle and adipose tissue (Galgani et al., 2008; Heilbronn et al., 2007; Storlien et al., 2004). Due to these implications, metabolic inflexibility is a concern as it can potentially lead to weight gain and the development of metabolic diseases (e.g. metabolic syndrome, insulin resistance, etc.) (Galgani et al., 2008; Storlien et al., 2004). However, it is still unclear whether it is metabolic inflexibility leads to or is an outcome of states such as obesity and insulin resistance (Heilbronn et al., 2007). Galgani et al. (2008), addresses that an individual's ability to adapt and oxidize fat appropriately in response to their daily diets will have lower muscle fat accumulation and be

less prone to insulin resistance, when compared to those less metabolically flexible.

Muoio (2014) best describes metabolic inflexibility as stiffness in mitochondrial substrate selection, leading to the inability to adjust to fuel types appropriately. Beyond muscular and adipose tissue, metabolic flexibility is significantly controlled at the cellular level by mitochondria. Acting as a gatekeeper of fuel metabolism, the mitochondria control the oxidation and break down of primary nutrients (primarily, fat and carbohydrate) into acetyl-CoA before entering into the citric acid cycle (Muoio, 2014). This mitochondrial substrate utilization can be predicted by the respiratory quotient (RQ) value. RQ oscillates between values of 0.70 (fat oxidation) and 1.0 (glucose oxidation), and can be used as an indicator of whole body fuel utilization (Galgani et al., 2008; Kelley, 2005; Muoio, 2014). RQ is a widely accepted value to measure metabolism before and after a stimulus (i.e. it is often used to assess metabolic flexibility) (Clevenger et al., 2014; Frayn, 1983; Stull et al., 2010; Weyer, Snitker, Bogardus, & Ravussin, 1999). RQ is measured by the whole body difference in oxygen consumption and carbon dioxide excretion. The inability of the value to shift in response to a fuel source is indicative of metabolic inflexibility (Galgani et al., 2008; Muoio, 2014). In addition to using RQ as a value to determine fuel oxidation, the calculation of substrate oxidation is also used. Frayn (1983) (Fat Oxidation= $1.67 V_{O_2} - 1.67 V_{CO_2} - 1.92 n$; Carbohydrate Oxidation = $4.55 V_{CO_2} - 3.21 V_{O_2} - 2.87 n$) validated two equations that accurately predict whole body glucose and lipid oxidation, accounting for protein metabolism. These equations

are also based upon oxygen and carbon dioxide values measured in vivo. With this measure, one has the ability to calculate whole body glucose and lipid metabolism of an individual in the number of grams metabolized per minute (Frayn, 1983).

Typically, as fat oxidation increases, glucose metabolism is inversely suppressed (Muoio, 2014). While skeletal muscle, adipose tissue, and mitochondria are frequently able to handle fuel partitioning during shortened feeding portions, it is the oversupply of nutrients, often found in the typical western diet, when this inflexibility is most prominent. In the case of chronic over-feeding, fuel switching becomes blunted (Muoio, 2014). Muoio (2014), describes this state of substrate rivalry as mitochondrial indecision, and acknowledges that it has been identified in many disease states such as obesity, Type II Diabetes, heart disease and physical inactivity. This mitochondrial indecision is commonly associated with other metabolically-important cellular and tissue functions such as insulin action, glucose disposal, lipolysis, lipid storage, cardiac contractility, and inflammatory response. In addition, evidence of mitochondrial indecision is evident at the level of gene and protein expression (Muoio, 2014).

Weight Status Disparities

While the prevalence of obesity in America has started to stabilize, compared to the growth last few decades, the number of American's with a body mass index (BMI) placing them into an overweight (≥ 25) or obese (≥ 30) category is still a staggering statistic (Ogden et al., 2006; Ogden et al., 2015; Ogden,

Carroll, Kit, & Flegal, 2014). Reports indicate that American adults over the age of 20 classified as overweight or obese is 68.5% and 36.5%, respectively. Reports also show similar statistics for all adult American women with 66.5% and 38.3% falling into the overweight or obese categories, respectively (Ogden et al., 2006; Ogden et al., 2015; Ogden et al., 2014).

There are many factors that play into weight gain and the prevalence of obesity, with many of these factors associated to lifestyle habits such as excessive caloric intake and poor dietary choices (Muoio, 2014). In fact, high fat, energy dense foods are a staple of the typical western diet. High fat diets can lead to weight gain; thus, contributing to the ongoing obesity epidemic (Clevenger et al., 2014). Obesity is a rising concern globally, and combatting this effect has been a major focus in the United States. Due to the number of health implications associated with excess weight gain, it is a serious public health concern.

Excessive weight gain contributes to inflammation, insulin resistance, glucose intolerance, and hyperinsulinemia, all of which negatively affect health and collectively contribute to the metabolic syndrome (Ogden et al., 2014). The inflammation and insulin resistance often associated with excessive weight gain, or obesity, are known indicators of a variety of disease states such as metabolic and cardiovascular. Along with the apparent rise in obesity, the rate of these disease states in the United States has also increased (Ogden et al., 2015).

In regards to weight status, previous research comparing lean and obese subjects has shown normal basal fatty acid uptake, but lower fatty acid oxidation and elevated storage in obese individuals (Heilbronn et al., 2007). Additionally,

metabolic flexibility is impaired in obese and weight-reduced obese women at a whole body level compared to their lean counterparts (Heilbronn et al., 2007). In morbidly obese women, fat oxidation levels have been reported to be as much as 50% lower than lean (Heilbronn et al., 2007). Storlien et al. (2004) notes that in response to a hyperinsulinaemic euglycaemic clamp, lean participants have been found to have a decrease in fatty acid uptake, with indicated changes in fat oxidation and storage, and a notable blunted response in the obese population as measured by a hyperinsulinaemic euglycaemic clamp. Additionally, an RQ value shift of 0.82 to 1.00 was seen in lean participants, but an RQ of 0.90 remained in obese participants, indicating metabolic inflexibility (Storlien et al., 2004). While much of the research shows a disparity in metabolic flexibility between lean and obese subjects, it has also been acknowledged that there is a range in metabolic flexibility even among healthy individuals (Berk et al., 2006).

Ethnic Disparities

While overweight and obesity trends are higher among women compared to men, there is still a drastic interracial difference, particularly between AA and CA women. For example, the prevalence of overweight and obesity among AA women is much higher (82.1% overweight and 56.7% obese) compared to CA women (64.6% overweight and 33.7% obese) (Ogden et al., 2015; Ogden et al., 2014).

The disparities in overweight and obesity prevalence among AA women are well-established and have become of major concern itself due to the linked health concerns. AA women, alongside higher obesity rates, also have a greater

increased risk of developing inflammatory related chronic diseases such as coronary heart disease and metabolic syndrome (Malayala & Raza, 2016; Weyer et al., 1999). Generally, AA women have a greater predisposal to metabolic syndrome due to higher insulin resistance, compared to other ethnic groups (Malayala & Raza, 2016). Malayala and Raza (2016) assessed health history of AA women from 2007 – 2011 and reported that 47% of AA women had at least three components of metabolic syndrome, with 14% possessing all five major components of metabolic syndrome (elevated abdominal obesity, triglyceride, HDL cholesterol, blood pressure, and/or fasting blood glucose levels). Ervin (2009) reported that AA women were found to be 1.5 times more likely to meet the criteria of metabolic syndrome compared to CA women. Additionally, Ervin (2009) reports higher prevalence in abdominal obesity (76.3% vs. 58.0%), increased blood pressure (53.4% vs. 33.0%), and elevated fasting glucose (38.7% vs 28.7%) in AA compared to CA women, respectively.

Beyond weight status, there is potential disparity in AA women in regards to metabolic flexibility as well. Previous research has shown AA women not only have a reduced fatty acid oxidation, but also a reduced metabolic rate when compared to CA women (Berk et al., 2006; Weyer et al., 1999). Branis, Etesami, Walker, Berk, and Albu (2015) found that metabolic flexibility was not altered during an hyperinsulinemic clamp in AA and CA women who had consumed either eucaloric (calories for meal controlled for based on participant's caloric needs) one week high fat or 1 week low fat diets. They did find, however, that AA women had a lower insulin clearance compared to CA women regardless of diet

(Branis et al., 2015). Berk et al. (2006) found AA women were unable to switch substrate use as efficiently as CA women after consuming a counterbalanced six day high fat diet and six day low fat diet. CA women increased fat oxidation from the low fat to high fat diets, while AA women did not. Additionally, while switching from the low to the high fat diets, CA women significantly decreased their carbohydrate oxidation, with AA women increasing carbohydrate oxidation. The CA women having had higher fat oxidation during the high fat diet and higher carbohydrate oxidation during the low fat diet indicates a proper response and substrate sensitivity (Berk et al., 2006; Muoio, 2014). Interestingly, there were no significant differences in fat or carbohydrate oxidation between ethnicities at baseline. Berk et al., 2006 acknowledges that this impaired metabolic inflexibility in AA women may be a factor contributing to the increased prevalence of disease.

Current literature suggests a potential difference in metabolic flexibility in AA women when compared to CA women. Stull et al. (2010) looked at both male and female participants of different ethnicities. They found that when fed a normal macronutrient balanced meal (50% carbohydrate, 35% fat, 15% protein) after nighttime fast there was increased metabolic flexibility and fat metabolism in AA compared to CA Americans. Insulin sensitivity was measured using a hyperinsulinemic clamp with RMR and RQ/substrate utilization measured using indirect calorimetry. These findings held true even when insulin sensitivity and presence or absence of Type II Diabetes were controlled for (Stull et al., 2010). Gerhard et al. (2000) had AA and CA women under go both a controlled

counterbalanced 4-week low fat and high fat diet. At the end of diet period, 24-hour response to a standard high fat meal (50% fat) was tested. They found that at both baseline and postprandially, there were no significant differences between ethnicities in response to the high fat meal after either dietary period.

Metabolic inflexibility, in response to a typical high fat Western diet, may be a mechanism contributing to the higher incidence of metabolic disease and obesity among women, and more specifically, AA women. While reduced fat oxidation in AA women has been shown, the impact that inflammation and gene expression in regards to metabolic flexibility after a single high fat meal within these populations has not been studied (Berk et al., 2006; Stull et al., 2010). Understanding how women of different ethnicities respond to these types of meals is important in further understanding potential mechanisms behind reduced fat oxidation and the higher prevalence of diseases in AA women.

While current literature shows the effects on metabolic flexibility after a high fat meal after 1-4 weeks of a controlled diet, it lacks the understanding on how women of different ethnicities respond to only a single high fat meal (Berk et al., 2006; Gerhard et al., 2000). AA women have a higher predisposal to certain disease states like obesity, coronary heart disease and metabolic syndrome (Malayala & Raza, 2016; Weyer et al., 1999). However, the connection between ethnicity and metabolic flexibility in response to a single high fat meal, (similar to that which is typically consumed in a Western diet), between AA and CA women has not been studied.

The increased prevalence of inflammation linked to obesity and metabolic diseases in AA women is also a concern. It is not clearly defined whether or not metabolic inflexibility in AA women could be a factor leading to this greater predisposal of disease.

Apolipoproteins and Cardiometabolic Health

Apolipoprotein-A1 and Apolipoprotein-B are the two of the most common proteins associated with both cholesterol and cardiometabolic health.

Apolipoprotein-A1 is the protein expression in relationship with the development of high density lipoproteins (HDL), or what is frequently coined as “good” cholesterol. A decrease in expression of HDL has been linked to metabolic syndrome, Type II Diabetes and other cardiometabolic diseases (Gerhard et al., 2000) . It has been shown that individuals deficient in the expression and development of Apolipoprotein-A1 fail to develop normal HDL molecules (Zannis, Chroni, & Krieger, 2006).

Apolipoprotein-B has been characterized as one of the key sources of the development of low density lipoproteins (LDL), or “bad” cholesterol. Additionally, it has been identified as one of the major players in the development of atherosclerosis (Shapiro & Fazio, 2017). Research has shown that the development of cholesterol rich Apolipoprotein-B containing proteins plays a proven and fundamental role in atherosclerosis and cardiovascular diseases (Shapiro & Fazio, 2017). It is due to these relationships seen between Apolipoprotein-A1 and Apolipoprotein-B that we have decided to assess these as potential indicators of metabolic health seen within our population.

The increased expression of LDL and decreased expression of HDL often seen in cardiometabolic disease states is often seen as a result of increased fat intake. As previously addressed, these diets are typical of that found in a Western diet, with related increases seen in prevalence of related metabolic diseases. Additionally, the role of these lipoproteins in regards to cholesterol in response to a single high fat meal could highlight some of the disparities of disease states such as cardiovascular diseases seen between CA and AA women (Ervin, 2009; Ogden et al., 2015).

Inflammation, Protein Levels and Gene Expression

Metabolic inflexibility can be seen all the way down to the molecular level (Calcada et al., 2014). This includes inflammation states and expression of genes associated with fat metabolism (Muoio, 2016; Calcda et al., 2014). The levels of circulating pro-inflammatory proteins in the body is linked with overall metabolic health (Phillips & Perry, 2013). Compared to CA, AA women have a higher predisposition to diseases that are often associated with chronic inflammation and metabolic dysfunction (Malayala & Raza, 2016; Weyer et al., 1999). Carroll et. al (2009), found that despite similar, or lower, amounts of visceral adipose tissue, AA women had higher concentrations of inflammatory biomarkers, such as IL-6.

Purpose

Due to the relationship of impaired metabolism and risk for future metabolic and cardiovascular diseases among obese and AA populations, studies designed to investigate the differences in metabolic response of a single high fat meal among CA lean, CA overweight/obese, AA lean, and AA overweight/obese

women are warranted. The primary purpose of this study is to assess the metabolic differences lean and overweight/obese women. Additionally, it is unclear whether metabolic inflexibility among AA women could be a factor leading to increased risk for future metabolic and cardiovascular disease. Thus, the secondary purpose of this project is to determine role of ethnicity on metabolic flexibility in response to a single high fat meal.

Chapter 3. Methods

Study Design

Participants were recruited to complete two study visits. The first visit was conducted to test participant's metabolic health in response to a single high fat meal. A second study visit was conducted to assess participant's fitness status and if there was a relationship between metabolic health and individual fitness levels.

Participant Demographics

Twenty-six Caucasian (CA) (n = 15) and African American (AA) (n = 11) women participated in this study (Table 1). To investigate the impact of ethnicity on metabolism, participants were categorized as CA or AA, as they identified as CA or AA, respectively. To investigate the impact of weight status, participants were categorized as lean ($BMI < 25$) or Overweight/Obese ($BMI \geq 25$). Additionally, participants were placed into groups based upon both ethnicity and weight status (CA lean, CA overweight/obese, AA lean, or AA overweight/obese). All study procedures were approved by the Western Kentucky University Institutional Review Board (IRB ID 17-021) (Appendix A). Participants were all healthy individuals with no underlying health conditions that could affect metabolism or put them at increased risk during exercise, as assessed by a Medical History Questionnaire and the Physical Activity Readiness Questionnaire (PAR-Q) (Appendix B).

	All	Lean	Overweight/Obese	
All	n = 27	n = 13	n = 14	p - value
Age	26.48 ± 6.00	25.15 ± 4.88	27.71 ± 6.82	0.276
BMI	29.66 ± 9.91	22.52 ± 1.56	36.28 ± 9.80	0.000
SBP	121.00 ± 12.83	118.16 ± 10.17	123.83 ± 14.00	0.289
DBP	75.92 ± 10.59	74.17 ± 5.57	74.17 ± 5.57	0.43
Glucose	100.37 ± 14.60	101.07 ± 12.88	99.71 ± 16.49	0.814
African American	n = 12	n = 5	n = 7	p - value
Age	26.75 ± 6.65	23.00 ± 4.24	29.43 ± 7.00	0.100
BMI	28.32 ± 6.91	22.29 ± 1.63	32.64 ± 5.82	0.003
SBP	121.2 ± 15.66	119.50 ± 10.34	122.33 ± 19.33	0.797
DBP	74.80 ± 14.31	75.00 ± 5.35	74.67 ± 18.75	0.974
Glucose	98.08 ± 15.1	103.00 ± 14.54	94.57 ± 15.64	0.366
Caucasian American	n = 15	n = 8	n = 7	p - value
Age	26.27 ± 5.65	26.5 ± 5.01	26.00 ± 6.7	0.872
BMI	30.72 ± 11.92	22.67 ± 1.61	39.93 ± 11.98	0.009
SBP	120.86 ± 11.02	117.50 ± 10.73	125.33 ± 10.59	0.199
DBP	76.71 ± 7.42	73.75 ± 5.99	80.67 ± 7.74	0.083
Glucose	102.2 ± 14.08	99.88 ± 12.62	104.86 ± 16.83	0.524

Table 1. Participant Characteristics. P-value indicates lean vs overweight/obese comparison.

Metabolic Flexibility Testing

All participants reported to the Western Kentucky University (WKU) Health Science Complex Exercise Physiology Laboratory. Prior to their testing session all participants were provided with specific written pre-testing instructions for the night before. This included a pre-testing dinner and snack that were standardized (50% carbohydrates, 30% fats, and 20% protein) and calorically-controlled based upon BMI adapted from pretest meals created by Registered Dieticians at the Washington University School of Medicine's Metabolic Kitchen as part of the Clinical Research Unit (lean: 800 calories; Overweight: 1000

calories; Obese: 1200 calories) (Appendix C). All participants were instructed to consume their pre-testing meals at approximately 6:00 pm the night before, and the pre-testing snack at 9:00 pm and all eating to be completed by 10:00 pm. Once the pre-testing meal and snack were consumed, participants were asked to fast for at least 10 hours, only consuming water, before the following morning's testing session. Participants were asked to stay away from mints, gum, or any unnecessary medications that could affect metabolism the morning of testing. All testing sessions were conducted first thing in the morning after the nighttime fast. Fasting was done to ensure an accurate measure of baseline blood markers, glucose, and resting metabolic rate measurements.

Upon arrival to the testing session, all participants were taken through the testing protocol and consented using an informed consent document approved by Western Kentucky University International Review Board. Once consent was obtained, participant filled out a medical health history questionnaire.

Participant's demographics were then taken. Height, weight and waist to hip measurements were taken. Body composition was determined by 7-site Jackson-Pollock skin fold measurements and was taken with a caliper (Harpender Skinfolds Caliper, Baty International, United Kingdom).

Once participant's demographics were obtained, participants underwent an initial baseline resting metabolic rate (RMR) measurement. Baseline (0 minutes, T1) RMR and respiratory quotient (RQ) (were measured for ~15 minutes using the TrueOne Canopy Option and TrueOne Metabolic Cart (TrueOne 2400, Parvomedics, Sandy, UT). Lipid and carbohydrate oxidation rates were

calculated by measurement of oxygen consumption and carbon dioxide production as previously described (Frayn, 1983). In order to measure resting metabolic rate, the participant was asked to lie comfortably in a supine position on a plinth. A canopy/hood-like device was placed over their head. This canopy was then connected to the metabolic cart so that inspired oxygen and expired carbon dioxide could be measured. The canopy was properly placed and the edges tucked in, to obtain a proper seal, which ensured all oxygen went into the tube and did not leak. Participants were instructed to remain still during testing, to breathe normally, not to talk, and not to fall asleep.

After the baseline resting metabolism measurement, a baseline blood pressure, heart rate and blood draw (0 minutes, T1; 8mL) were obtained. Blood was drawn with a butterfly angiocath in the AC (bend of the arm) or in the hand. It was flushed with saline to maintain patency and secured and kept in for four hours during the remaining of the study period. All study procedures and venous blood draws were supervised by a doctor of nursing practice or a trained physician. Additionally, baseline glucose and lactate measurements were taken alongside the blood draw.

After the baseline blood draw, participants consumed a standard 1062-kcal meal that is high in fat, similar in composition to previous studies (Heilbronn et al., 2007; Jakulj et al., 2007). The high fat meal consisted of the “WKU study smoothie” made from Smoothie King (Table 2, Table 3). This smoothie is similar in fat content to an average high fat meal found in the standard western diet. Participants were encouraged to consume the shake within 30 minutes.

	Nutty Super-grain Smoothie (Smoothie King Inc.)	2 Peanut Butter	1 Almond	1 Cocoa	Total (g)
Fat (g)	21	36	8	1	66
Saturated Fat (g)	3	5	1	0.5	9.5
Carbohydrates (g)	54	16	4	4	78
Sugar (g)	30	2	1	0	33
Protein (g)	18	16	4	1	39
Fiber (g)	7	6	2	2	17

Table 2. Nutritional Break Down of High Fat Shake.

	Total (g)	Calories (kcal)	Percent of Shake Calories
Fat (g)	66	594	55.93%
Carbohydrates (g)	78	312	29.38%
Protein (g)	39	156	14.69%
Total		1062	100%

Table 3. Caloric Break Down of High Fat Shake.

Additional blood samples, lactate, and glucose measurements were taken from the same angiocath at 120 (T2; 8mL), and 240 (T3; 8 mL) minutes after the high fat meal was consumed. Resting metabolic rate and respiratory quotient were obtained a second time for ~15 minutes, at ~120 minutes (T2) and from minute 210 to 240 (T3) minutes post high fat load (Illustration 1). A total of 24mL blood was drawn. Additional post-testing blood pressure and heart rate were taken as well. At the end of the study, the angiocath was removed by the nurse practitioner or doctor. Time points for assessment of metabolic responses to a high fat load were chosen based on data from previous studies exploring metabolic inflexibility in other populations (Heilbronn et al., 2007).

During the four-hour study period, participants were asked to remain reclined and resting. They completed surveys during this time, including the

National Institutes of Health's validated Dietary History Questionnaire II to determine potential differences in normal diet and the International Physical Activity Questionnaire to determine self-recall of average weekly physical activity levels. Additionally, upon completion of the testing session participants were given an Actigraph accelerometer to wear for the 7 days following to determine average weekly activity.

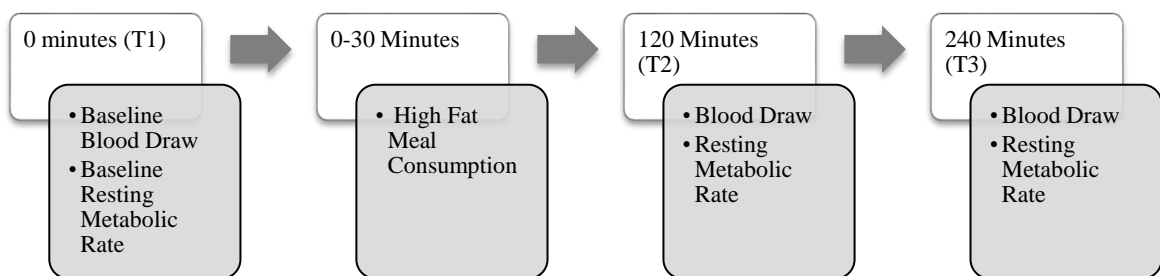


Illustration 1. Timeline of Metabolic Flexibility Testing Session.

Blood analysis

At all time periods, blood was collected into EDTA and Lithium Heparin tubes. 800ul of whole blood was immediately placed into 900ul of RNAlater (Thermo Fisher Scientific) and stored at -20C. Samples were spun down for 15 min and plasma samples were aliquoted and stored at -80C. Plasma samples were measured for HDL, LDL and insulin levels (Analox GM4, Analox Solutions). 500 uL of whole blood and RNAlater mixture was used to isolate RNA using the TRIzol reagent and reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcript Kit (Thermo Fisher Scientific). Additionally, Apolipoprotein-A1 and Apolipoprotein-B were measured (Diazyme

Laboratories). All experiments done following instructions provided by the supplier.

Statistics

Statistical analyses were done using SPSS statistics software. Fold changes were calculated for and assessed to indicate the up or down regulation of metabolism and protein changes in response to a single high fat meal. Fold changes were calculated by dividing T2 (120 min) oxidation values by T1 (0 min) oxidation values (T2/T1) to assess the overall change in fat or carbohydrate oxidation and Apolipoprotein-A1 and Apolipoprotein-B expression between these same time points. Additional fold changes were calculated between T1 and T3 for Apolipoprotein-A1 and Apolipoprotein-B (T3/T1). For all analyses, if statistical assumptions of normality were not met, variables were log transformed for analysis. Baseline characteristics for all groups were compared using unpaired t-tests and the effects of time were assessed using pair-samples t-tests. Data are presented as means \pm SD.

In order to determine differences in *metabolic* measures (i.e. fat and carbohydrate oxidation) and *biochemical* levels (Apolipoprotein-A1 and Apolipoprotein-B) differences among women, across time, data were assessed using repeated-measures ANOVA with emphasis on a *time* (T1=0 vs. T2=120 vs. T3=240 min) X *BMI* (lean vs. overweight/obese) X *ethnicity* (AA vs. CA) comparisons. Comparisons between lean and overweight/obese women were performed with repeated-measures ANOVA with emphasis on a *time* (T1=0 vs. T2=120 vs. T3=240 min) X *BMI* (lean vs. overweight/obese) interaction in terms

of *biochemical* (i.e. Apolipoprotein-A1 and Apolipoprotein-B) and *metabolic measures* (i.e. fat and carbohydrate oxidation) to assess differences in lean and overweight/obese women in response to the high fat meal. Additionally, comparisons between CA and AA women were performed with repeated-measures ANOVA with emphasis on a *time* (T1=0 vs. T2=120 vs. T3=240 min) X *ethnicity* (AA vs. CA) interaction in terms of *biochemical* (i.e. Apolipoprotein-A1 and Apolipoprotein-B) and *metabolic measures* (i.e. fat and carbohydrate oxidation) assessing CA and AA women's response to a single high fat meal.

Additionally, when significant interactions were seen for *time X ethnicity X BMI*, *time X BMI*, or *time X ethnicity*, for *biochemical* and *metabolic measures*, follow up tests were subsequently performed to further assess the impact of these measures. Independent samples t-test and/or paired t-tests were performed for further analysis between all groups, when appropriate (e.g. CA lean vs. CA overweight/obese, CA lean vs. AA lean, CA lean vs. AA overweight/obese, CA overweight/obese vs. AA lean, and CA overweight/obese vs. AA overweight/obese). When applicable, post hoc comparisons were performed with contrast-contrast analyses.

Chapter 4. Results

Fat and Carbohydrate Oxidation

Overall, there were significant *time* and *BMI* effects ($p<0.05$) for fat and carbohydrate oxidation in response to the high fat meal. Fat oxidation was increased significantly from T1 to T2 (T1: 0.08 ± 0.03 vs. T2: 0.10 ± 0.04 ; $p<0.01$), but there was no additional increase in fat oxidation from T2 to T3 (T2: 0.10 ± 0.04 vs. T3: 0.11 ± 0.01 ; $p=0.42$). Carbohydrate oxidation was increased significantly from T1 to T2 (T1: 0.47 ± 0.01 vs. T2: 0.57 ± 0.02 ; $p<0.01$), but there was no additional increase in carbohydrate oxidation from T2 to T3 (T2: 0.57 ± 0.02 vs. T3: 0.58 ± 0.03 ; $p=0.07$). Regarding weight status, the overall difference between the lean and overweight/obese in regards to absolute fat oxidation measures approached significance ($p=0.08$), while there was a significant difference for carbohydrate oxidation ($p<0.01$). Additionally, there was a significant *time* X *ethnicity* interaction ($p=0.04$) for fat and carbohydrate oxidation in response to the high fat meal across the three time points.

Fat Oxidation

Overall

When fat oxidation was assessed independently, from T1 to T2, there was a significant *time* effect ($p<0.01$) and a significant *time* X *ethnicity* interaction ($p<0.02$) in response to the high fat meal. Pairwise comparisons revealed a significant increase in fat oxidation for *time* ($p<0.01$) and *BMI* ($p=0.02$). There was a significant increase in fat oxidation from T1 to T2 was seen among all women (CA lean: $57.9\pm24.5\%$ increase; $p<0.01$; CA overweight/obese:

30.2±11.8% increase; $p<0.01$; AA lean: 10.2±18.1% increase; $p<0.01$, AA overweight/obese: 40.6±52.6% increase; $p<0.01$) (Figure 1).

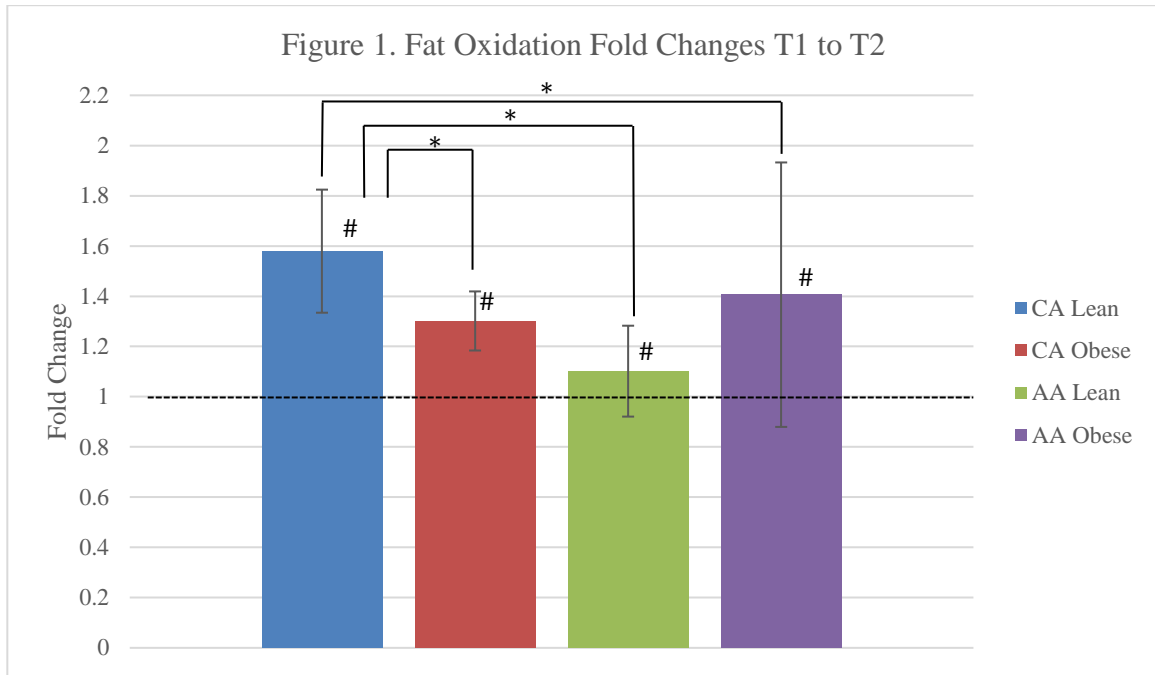


Figure 1. Dashed line represents baseline values (T1 – 0 min). Fat Oxidation Fold Changes from 0 (T1) to 120 (T2) minutes (calculated by T2 / T1). # $p<0.05$ significant change from T1; * $p<0.05$ significance between groups.

Impact of Ethnicity and Weight Status

When considering the impact of both ethnicity and weight status on fat oxidation, CA lean women displayed a significantly higher fold change (T2/T1) for fat oxidation in response to the high fat meal compared to AA lean women (CA lean: 1.57±0.25 fold vs. AA lean: 1.10±0.18 fold; $p<0.01$) (Figure 1). Additionally, CA lean fat oxidation fold change in response to the high fat meal was significantly higher when compared to AA overweight/obese women (CA lean: 1.57±0.25 fold vs. AA overweight/obese: 1.41±0.53 fold; $p=0.01$) (Figure 1).

Among the lean women, there were no significant differences between CA and AA in fat oxidation at T1 (CA lean: 0.05 ± 0.02 vs. AA lean: 0.07 ± 0.02 ; $p=0.13$) or T2 (CA lean: 0.08 ± 0.02 vs. AA lean: 0.84 ± 0.03 ; $p=0.97$). Among the overweight/obese women, there were no significant differences between the CA and AA in fat oxidation at T1 (CA overweight/obese: 0.10 ± 0.04 vs AA overweight/obese: 0.09 ± 0.04 ; $p=0.51$) or T2 (CA overweight/obese: 0.13 ± 0.05 vs AA overweight/obese: 0.11 ± 0.04 ; $p=0.37$).

Impact of Weight Status Within Ethnicity

When considering the impact of weight status within ethnicity, among the CA women only, lean women displayed a significantly higher increase in fat oxidation in response to the high fat meal compared to CA overweight/obese women (CA lean: 1.57 ± 0.25 fold vs. CA overweight/obese: 1.30 ± 0.12 fold; $p=0.03$) (Figure 1). There were no significant differences in the fold change (T2/T1) for fat oxidation between AA lean and AA overweight/obese women.

Absolute fat oxidation was significantly higher among the CA overweight/obese compared to the CA lean at T1 (CA lean: 0.05 ± 0.02 vs. CA overweight/obese: 0.10 ± 0.4 ; $p < 0.01$) and at T2 (CA lean: 0.08 ± 0.23 vs. CA overweight/obese: 0.13 ± 0.5 ; $p=0.03$) (Figure 2A). However, there were no significant differences according to weight status among the AA overweight/obese and the AA lean women in terms of fat oxidation at T1 (AA lean: 0.075 ± 0.02 vs. AA overweight/obese: 0.09 ± 0.04 ; $p=0.59$) or T2 (AA lean: 0.084 ± 0.037 vs. AA overweight/obese: 0.11 ± 0.04 ; $p=0.29$) (Figure 2B).

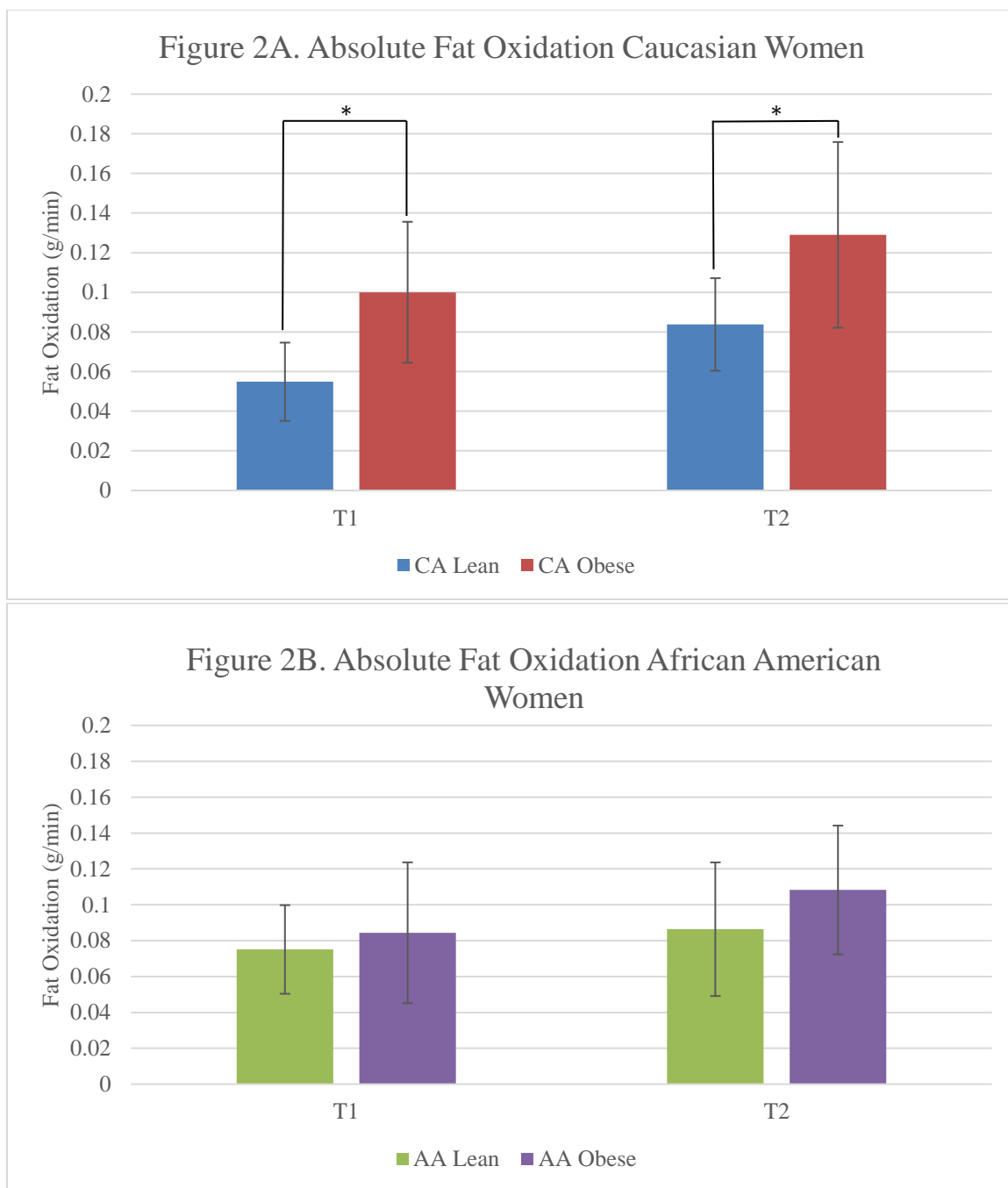


Figure 2. The absolute fat oxidation values for T1 and T2. * $p < 0.05$ significance between groups.

Carbohydrate Oxidation

Overall

When carbohydrate oxidation was assessed independently, from T1 to T2, there was a significant *time* effect ($p<0.01$). Pairwise comparisons revealed, among all women, a significant increase in the fold change for carbohydrate oxidation (T2/T1) from T1 to T2 ($+1.21\pm0.02$; $p<0.01$) (Figure 3). Between subjects comparisons revealed a significant *BMI* effect ($p<0.01$) for carbohydrate oxidation. Pairwise comparisons indicated that absolute carbohydrate oxidation among the overweight/obese group was significantly higher compared to the lean (lean: 0.46 ± 0.03 vs. overweight/obese: 0.59 ± 0.03 ; $p<0.01$). There was a trend for an *ethnicity X BMI* interaction effect ($p=0.07$).

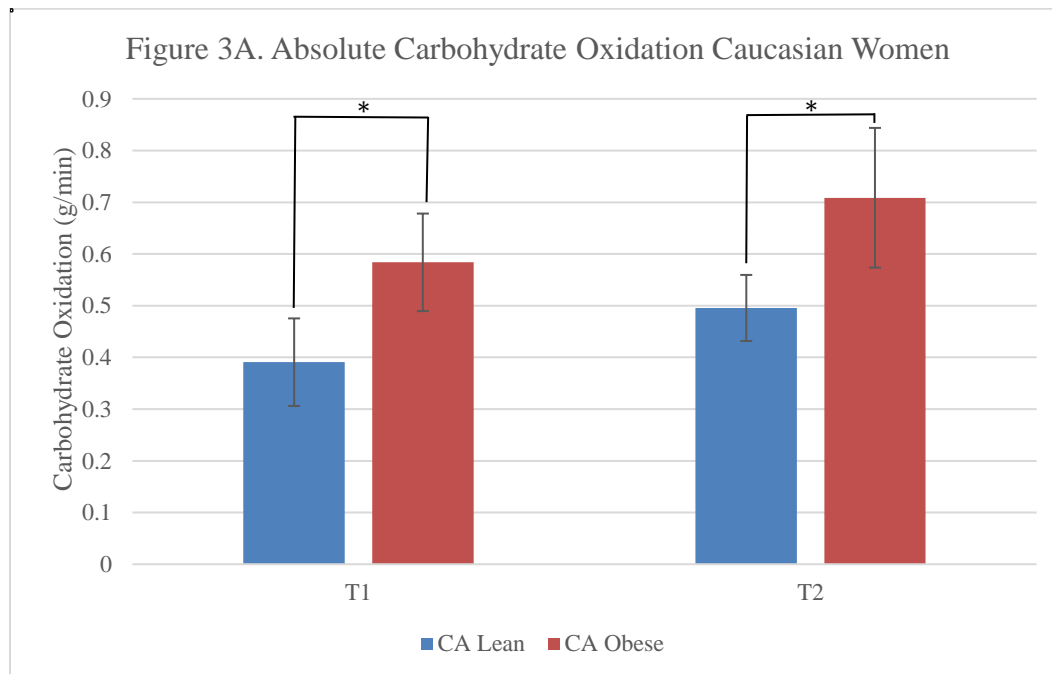
Impact of Weight Status and Ethnicity

There were no significant differences in the increase in carbohydrate oxidation (from T1 to T2) in response to the high fat meal according to ethnicity and weight status (CA lean: $29.2\pm13.60\%$ increase vs. CA overweight/obese: $21.1\pm7.40\%$ increase vs. AA lean: $17.3\pm8.80\%$ increase vs. AA overweight/obese: $23.0\pm13.30\%$ increase; $p=0.39$) (Figure 3). When assessing the impact of ethnicity within weight status groups, there were no significant differences between CA lean and AA lean in carbohydrate oxidation at T1 (CA lean: 0.39 ± 0.30 vs. AA lean: 0.43 ± 0.41 ; $p=0.41$) or T2 (CA lean: 0.49 ± 0.30 vs. AA lean: 0.50 ± 0.04 ; $p=0.91$). However, among the overweight/obese women only, there were significant differences between the CA overweight/obese and AA overweight/obese in carbohydrate oxidation at T2 (CA overweight/obese:

0.70±0.03 vs AA overweight/obese: 0.58±0.03; $p<0.05$) and a trend for a significant difference at T1 (CA overweight/obese: 0.58±0.03 vs. AA overweight/obese: 0.48±0.11; $p=0.06$) where carbohydrate oxidation was higher among the CA overweight/obese at both time points.

Impact of Weight Status Within Ethnicity

Among the Caucasian women only there was a significant difference in absolute carbohydrate oxidation according to weight status. Absolute carbohydrate oxidation was significantly higher among the CA overweight/obese compared to the CA lean at T1 (CA lean: 0.39±0.30 vs. CA overweight/obese: 0.58±0.03; $p<0.01$) and at T2 (CA lean: 0.49±0.30 vs. CA overweight/obese: 0.70±0.03; $p<0.01$) (Figure 3). However, there were no significant differences according to weight status among the AA overweight/obese and the AA lean women in terms of absolute carbohydrate oxidation at T1 or T2 (Figure 3).



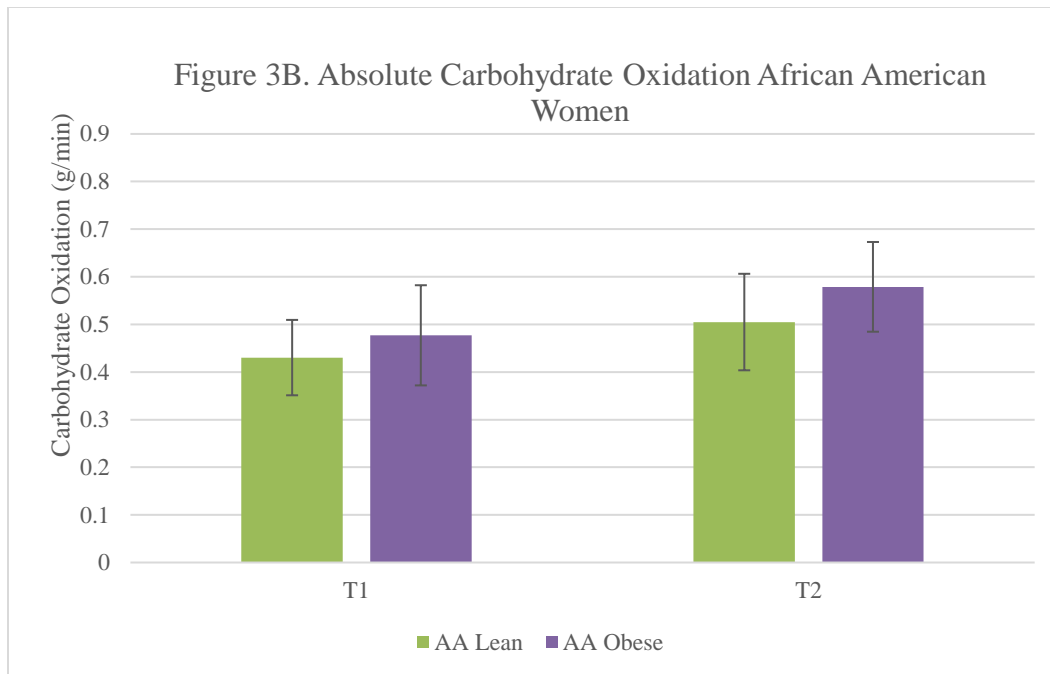


Figure 3. The absolute carbohydrate oxidation values for T1 and T2. * $p < 0.05$ significance between groups.

Apolipoprotein Expression

Overall

Overall, when assessed across all time points, there were no significant *time* effects seen for Apolipoprotein-A1 and Apolipoprotein-B ($p=0.38$). There was a between subjects *BMI* effect that approached significance ($p=0.07$). Additionally, there was a *time X ethnicity* interaction ($p=0.03$) and a *time X BMI X ethnicity* interaction that approached significance ($p=0.10$). Tests of within subject contrasts showed a trend for a *time X BMI X ethnicity* interaction for Apolipoprotein-A1 ($p=0.06$). Univariate tests indicated a *time X ethnicity* interaction for Apolipoprotein-B approaching significance ($p=0.08$). Tests of between subjects effects showed a *BMI* effect for Apolipoprotein-B ($p=0.03$).

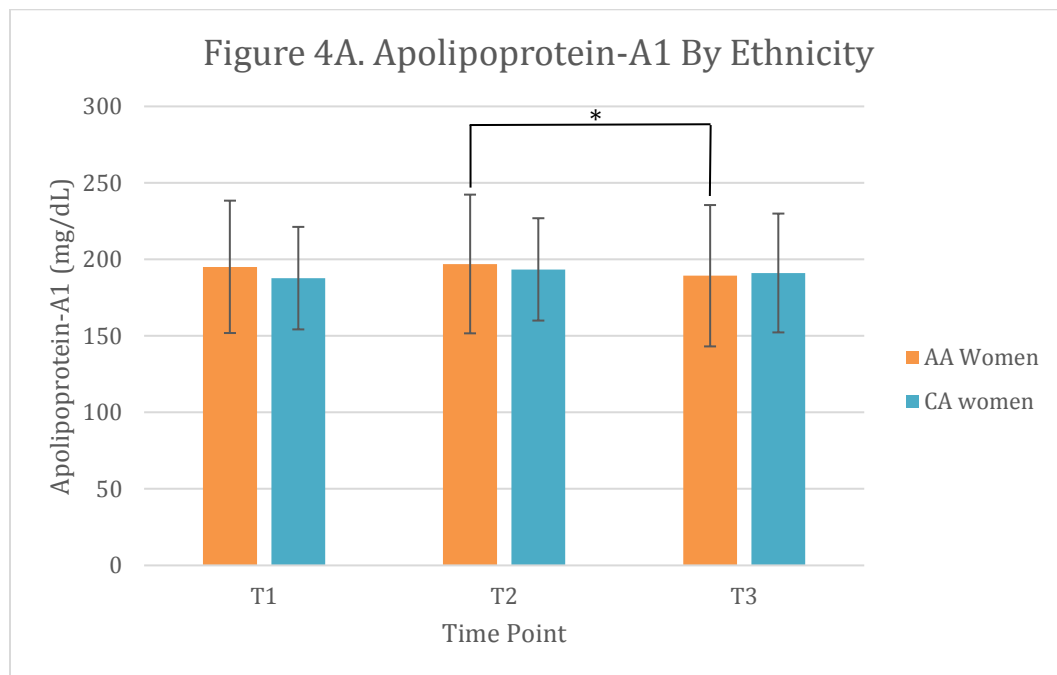
Apolipoprotein-A1

Overall

When assessed independently across all time points, there was no significant *time* effect for Apolipoprotein-A1 ($p=0.57$). Additionally, there were no significant *time X ethnicity* ($p=0.69$) or *time X BMI* ($p=0.59$) interaction effects. However, tests of within subject's contrasts revealed a *time X ethnicity X BMI* interaction that approached significance ($p=0.06$).

Ethnicity

When assessing the change in Apolipoprotein-A1 across time among the CA women only, there were no significant changes in Apolipoprotein-A1 protein across any of the time points. However, among the AA women only, there was a significant decrease in Apolipoprotein-A1 from T2 to T3 (T2: 197.01 ± 45.33 vs T3: 189.33 ± 46.20 ; $p=0.01$) (Figure 4).



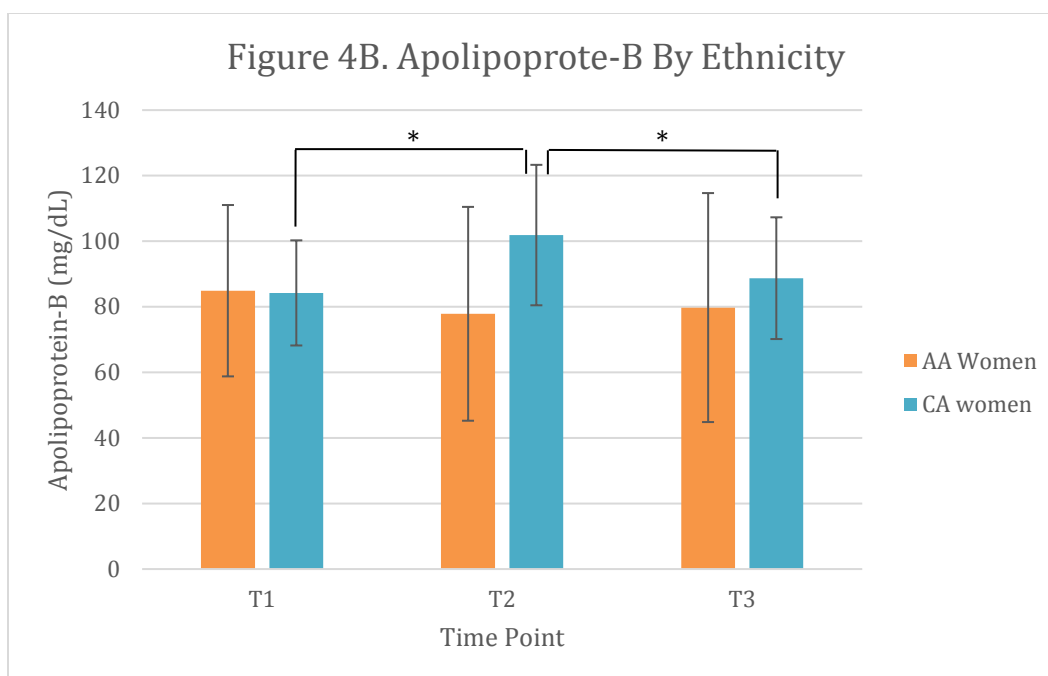


Figure 4. Apolipoprotein-A1 and Apolipoprotein-B over time for ethnicity.

* $p < 0.05$ indicates a significant change between time points.

Impact of Weight Status and Ethnicity

There was no significant difference in Apolipoprotein-A1 according to weight status (Figure 5). In terms of weight status comparisons within ethnic groups, there were significant differences in the change in Apolipoprotein-A1 among the CA women only from T1 to T3. Apolipoprotein-A1 decreased among the CA lean, but increased among the CA overweight/obese (CA lean: $-5.46 \pm 0.15\%$ decrease vs. CA overweight/obese: $+11.13 \pm 0.14\%$ increase; $p = 0.04$) (Figure 6). Weight status comparisons among the AA women only, revealed no differences between AA lean and AA overweight/obese women. In terms of assessing differences among ethnicity within weight status groups, there were no significant differences in Apolipoprotein-A1 protein levels between AA lean and

CA lean, nor were there any significant differences between AA overweight/obese and CA overweight/obese women.

Apolipoprotein-B

Overall

When assessing Apolipoprotein-B independently, tests of within subject's effects indicated a *time X ethnicity* interaction that approached significance ($p=0.08$). Additionally, tests of within subjects contrasts revealed a significant *time X ethnicity* contrast ($p=0.04$) and a *time* effect that approached significance ($p=0.08$). Tests of between subject's effects indicated a significant *BMI* effect ($p=0.03$). Pairwise comparisons indicated a significant *BMI* effect ($p=0.02$).

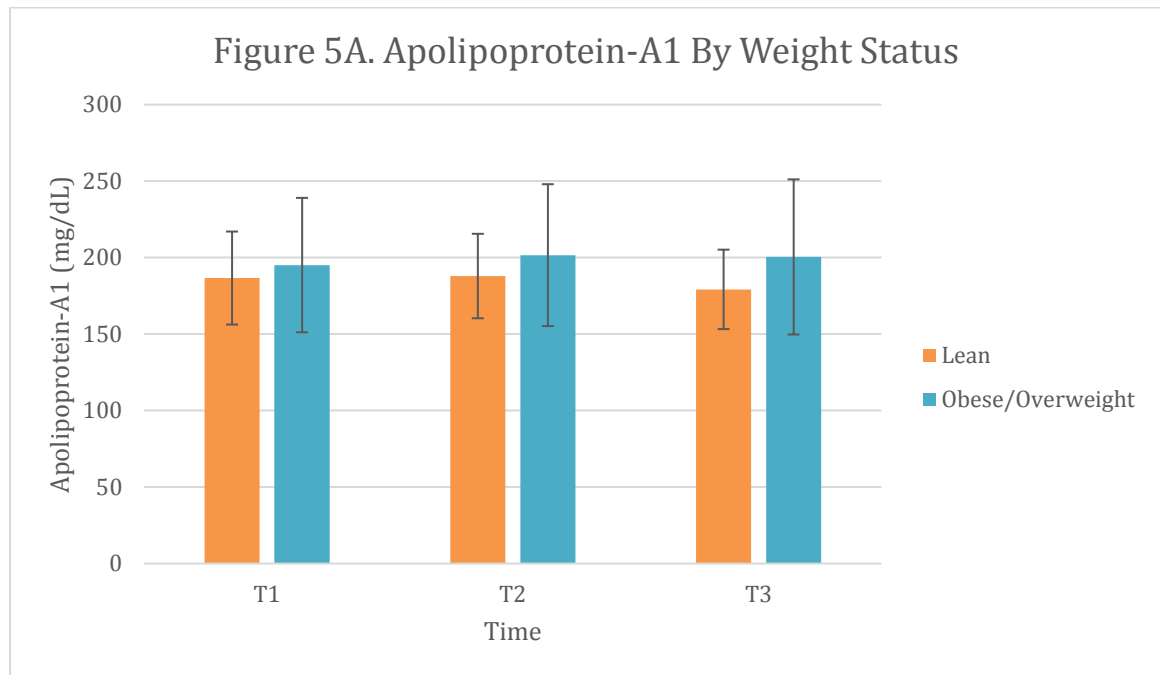
Ethnicity

When assessing the overall impact of ethnicity on Apolipoprotein-B protein levels, increased Apolipoprotein-B protein among CA women compared to AA women after consuming the high fat meal at T2 approached significance (CA: 105.0 ± 31.8 vs. AA: 83.4 ± 26.2 ; $p=0.06$) (Figure 4). Additionally, the change in Apolipoprotein-B from T1 to T2 approached significance, where Apolipoprotein-B increased among the CA women, but decreased among the AA women (CA: $+12.07 \pm 3.41\%$ increase vs. AA: $-0.80 \pm 2.02\%$ decrease; $p=0.07$). Paired samples t-tests revealed a significant increase in Apolipoprotein-B from T1 to T2 (T1: 88.1 ± 16.69 vs. T2: 105.03 ± 31.80 ; $p=0.05$) and a significant decrease in Apolipoprotein-B from T2 to T3 (T2: 105.03 ± 31.80 vs. T3: 91.58 ± 26.81 ; $p=0.02$)

among the CA women only (Figure 4). However, there were no significant changes in Apolipoprotein-B across these time points among the AA women.

Weight Status

Pairwise comparisons indicated that Apolipoprotein-B expression overall was higher among the overweight/obese women compared to lean (lean: 79.6 ± 5.95 vs. overweight/obese: 99.2 ± 5.57 ; $p=0.02$). When assessing the overall impact of weight status on differences in Apolipoprotein-B protein levels, there were trends for elevated Apolipoprotein-B protein among the obese women at all three measured time points: T1 (lean: 79.3 ± 20.1 vs. overweight/obese: 95.4 ± 24.3 ; $p=0.07$), T2 (lean: 84.7 ± 25.9 ; overweight/obese: 105.20 ± 30.46 ; $p=0.07$) and T3 (lean: 78.1 ± 24.7 vs. overweight/obese: 97.0 ± 25.4 ; $p=0.06$) (Figure 5).



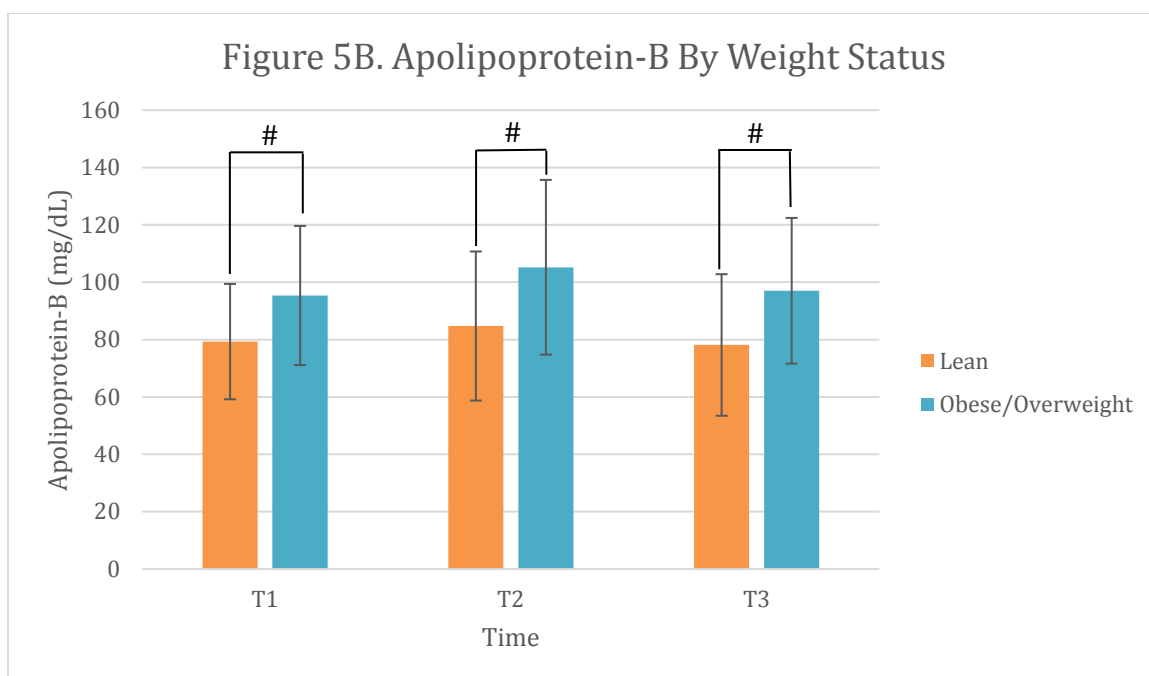


Figure 5. Apolipoprotein expression over time by weight status. # $p=0.07$

indicates a difference between groups approaching significance.

Impact of Ethnicity and Weight Status

In terms of assessing differences among ethnicity within weight status groups, there was a significant difference in Apolipoprotein-B protein expression at T2 between the overweight/obese women, where Apolipoprotein-B was significantly higher among the CA overweight/obese compared to the AA overweight/obese women (CA overweight/obese: 121.91 ± 34.6 vs. AA overweight/obese: 88.49 ± 12.54 ; $p=0.03$) (Figure 6). Additionally, the change from T1 to T2 between CA overweight/obese and AA overweight/obese women approached significance, where Apolipoprotein-B increased among the CA overweight/obese women, but decreased by among the AA overweight/obese women (CA overweight/obese: $+13.38 \pm 4.28\%$ increase vs. AA overweight/obese:

-3.62±2.1% decrease; $p=0.06$). However, among CA lean and AA lean women, there were no differences at any point.

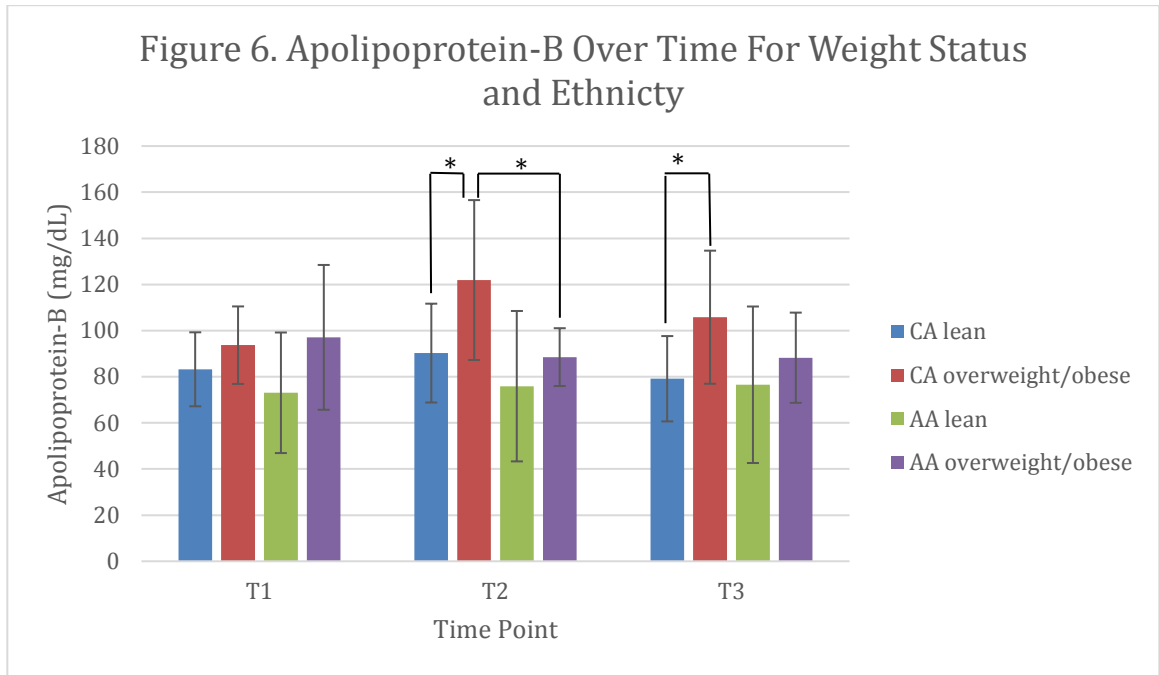


Figure 6. Apolipoprotein-B over time by over time for weight status and ethnicity. * $p<0.05$ indicates significant differences between groups.

Impact of Weight Status Within Ethnicity

When investigating the impact of weight status among the CA women only, data indicated that Apolipoprotein-B levels were significantly higher in CA overweight/obese compared to CA lean women at T2 (CA lean: 90.25±21.4 vs. CA overweight/obese 121.91±34.6 $p=0.05$) and T3 (CA lean: 79.13±18.56 vs. CA overweight/obese 105.80±28.85 $p=0.05$), but not at T1 (CA lean: 83.21±16.03 vs. CA overweight/obese 93.67±16.8 $p=0.24$) (Figure 7). Additionally, the change in Apolipoprotein-B from T1 to T3 approached significance (CA lean: -5.12±1.10% decrease vs. CA overweight/obese: +13.39±2.59% increase; $p=0.08$). However,

among AA women there were no differences according to weight status between AA lean and AA overweight/obese women at any time point.

Chapter 5. Discussion

The effects of a single high fat meal on metabolic response were assessed among women in this study. Due to ethnic disparities in metabolic diseases, as well as the disease states associated with increased weight status, we investigated this metabolic response in both lean and overweight/obese Caucasian (CA) and African American (AA) women. Overall, the results of this study suggest that overweight/obese women tend to respond less favorably to metabolic stimuli (i.e. a high fat meal), compared to lean women. Results also suggest that AA women have a decreased metabolic response, in terms of their ability to increase fat oxidation, after consuming a high fat meal compared to CA women.

Metabolism

In regards to weight status, there appears to be a discrepancy in regards to a shift in fat metabolism. Overall, there were no differences in the increase of fat metabolism between the CA and AA groups. However, when further assessing metabolic differences between weight status within ethnicity it appears that there are significant differences in fat metabolism apparent among weight status groups within ethnicity. For example, CA lean women were able to upregulate fat metabolism to a greater extent in response to high-fat meal compared to their CA obese counterparts; however, there were no differences between the AA lean and their AA obese counterparts.

Current literature supports weight status discrepancies between lean and obese women in terms of metabolic flexibility, with lean women having a greater increased metabolism than obese women. Storlien et al. (2004) looked at the

response to a hyperinsulinaemic euglycaemic clamp, while clamping encourages a carbohydrate response, their overall findings were similar. They measured metabolic response by detecting a shift in the respiratory quotient, rather than whole body substrate oxidation, and reported a significant metabolic shift towards carbohydrate metabolism lean participants and a blunted response in overweight/obese participants. Their findings indicate metabolic inflexibility among overweight/obese women (Storlien et al., 2004). This is similar to that of the findings we saw between our CA lean and CA overweight/obese women, with CA overweight/obese women having a decreased metabolic response to a single high fat meal (Figure 1). Tentolouris et al. (2011) found an increase in fat metabolism in response to a single high fat meal in all study participants (similar to the present study); however, they found no differences in macronutrient oxidation (i.e. fat and carbohydrate oxidation) between the two groups. Additionally, Tentolouris et al. (2008), found no macronutrient oxidation differences in lean and overweight/obese women after a high fat meal.

In regards to the impact of ethnicity on metabolic flexibility, there were no significant differences in fat metabolism between CA and AA women when grouped together irrespective of weight status. However, after consuming a high fat meal, CA lean women had significantly increased fat oxidation compared with both the lean and overweight/obese AA women (Figure 1). These findings are similar to reports by Branis et al. (2015). They found that after undergoing a high-dose insulin clamp on the seventh day of both a low and high fat diet, AA women had lower rates of postprandial fat oxidation. This supports our findings

of suppressed postprandial fat oxidation seen in our AA participants, compared to CA lean participants, in response to high fat feeding. Additionally, Berk et al. (2006) assessed the metabolic effect of switching between a 6 day high fat (50% fat) and 6 day low fat (30% fat) diets, followed by single high fat meal. Berk et al. 2016 reported overall significant differences between CA and AA women in regards to fat oxidation in response to the post diet high fat meal, with CA women having a greater increase in fat metabolism compared to AA. This is similar to our results in regards to the differences seen between CA lean and AA lean as well as CA lean and AA obese. However, unlike our study, they did not separate the ethnic groups into separate weight groups (Berk et al., 2006).

In regards to our study, this leads us to speculate that a healthy weight status itself, such as that seen in lean subjects, is enough to elicit improvements in metabolism among CA women, but not AA women. This could lend as a potential protective mechanism throughout life span among CA lean women, but not overweight/obese or AA women. This potential for lesser metabolic flexibility could possibly be a contributing mechanism of AA women's predisposal to develop cardiovascular and metabolic diseases. It may also suggest that controlling the amount of fat in the diet may be even more critical among AA women as they are going to be less likely to metabolize it efficiently.

Clevenger et al. (2014) assessed the changes in fat oxidation in three different (saturate, monounsaturated, polyunsaturated) isocaloric (consistent in calories content) high fat shakes (70% fat, 726-748 calories, 56-58 grams of fat), similar to ours (56% fat, 1,062 calories, 66 grams of fat). They found

comparable results to our own with a significant time effect on both lipid and carbohydrate oxidation rates postprandially, with a more drastic postprandial increase in fat oxidation. Additionally, they found that the type of fat made no difference in whole body substrate utilization among participants (Clevenger et al., 2014).

Overall there were significant differences between lean and overweight/obese women in regards to carbohydrate metabolism. Additionally, there were significant differences between CA lean and CA overweight/obese women in regards to carbohydrate metabolism, but not among AA lean and AA obese women. However, interestingly there were no metabolic differences seen between AA lean and AA overweight/obese women in regards to carbohydrate metabolism, similar to that of our findings for fat metabolism. Further research assessing the metabolic response of CA and AA, lean and overweight/obese, women in response to a single high carbohydrate meal may yield interesting results in comparison with the current findings of our and previous studies of the response to a single high fat meal.

Apolipoprotein-A1 and Apolipoprotein B

Overall, in regards to Apolipoprotein-A1 levels there were no ethnic differences in response to a single high fat meal. However, among CA women there were significant changes according to weight status, with CA overweight/obese significantly increasing Apolipoprotein-A1 expression from T1 to T3, with no differences among AA women (Figure 4). The findings of Wang et

al., (2014) show an increase in Apolipoprotein-A1 among AA women and a decrease in CA women after a one week standard diet and test meal (40% fat, 40% carbohydrate). However, Wang et al. (2014), controlled for insulin sensitivity as well as weight status factored in as visceral adipose tissue and its relationship with Apolipoprotein-A1 expression, which the present study did not. The control for insulin response its self could have potentially highlighted any further differences among women in regards to Apolipoprotein-A1 expression, or potentially yielded results similar to that seen in Wang et al. (2014).

In regards to Apolipoprotein-B, CA women had an increased expression in Apolipoprotein-B at T2, compared to AA women (Figure 4). Additionally, CA women showed a greater increase in Apolipoprotein-B expression compared to AA women from T1 to T2. As a whole, overweight/obese women showed elevated Apolipoprotein-B at all time points (Figure 5). Additionally, CA overweight/obese women showed an increase Apolipoprotein-B expression than that of CA lean, with no differences seen between the AA lean and AA obese groups. With Apolipoprotein-B being associated with development LDL molecules, or “bad” cholesterol, this itself could shed light on increased rates of LDL, elevated cholesterol, and cardiovascular related disease states among overweight/obese individuals (Shapiro & Fazio, 2017). However, this does not support the trends of AA women having an increased prevalence of cardiovascular diseases compared to that of CA women.

Our findings suggest that increased weight status its self may play a greater role in the response of Apolipoprotein-A1 and Apolipoprotein-B

expression after consuming a high fat meal among CA women, but not AA women. This is similar in trend to that of our current findings for fat and carbohydrate metabolism. Furthermore, future analysis in regards to cholesterol expression, lipoproteins, insulin response and metabolic health may be necessary to better evaluate differences among these groups.

Limitations

A potential limitation of our study was that our meal was isocaloric, rather than eucaloric in regards to participants resting metabolic rate or weight status. This may have provoked a greater fat stimulus in lean participants, when compared to the overweight/obese women, who were larger in weight status and had greater resting metabolic rates. However, this does not appear to affect the results in regards to the comparison of women by weight status (CA lean vs AA lean women; CA overweight/obese vs AA overweight/obese women), who have no notable weight status and had no or resting metabolic rate differences within groups. This suggests that the differences in metabolism between CA lean and AA lean women are still an interesting find. Additionally, the lack a difference in metabolic response found between CA overweight/obese and AA overweight/obese women are still supported by our findings, despite this potential limitation.

Another potential limitation is that our participants did not have a controlled diet beyond the pre-testing dinner. This meal was standard among all women to best control the participant's metabolic state prior to the metabolic testing session. While other studies controlled diet for 1-4 weeks before testing (Berk et al., 2006;

Branis et al., 2015; Gerhard et al., 2000), our study focused on the acute metabolic response to a single high fat meal. This is often reflective of the typical American diet, and is not fully understood whether a single high fat meal is enough to elicit the negative effects seen with metabolic syndrome, such as decreased metabolic flexibility.

In regards to Apolipoprotein results, previous studies had controlled for insulin and weight status. Additionally, previous studies had measured and reported the insulin response to a single high fat meal. The lack of data in regards to post prandial insulin response to that of a high fat meal is a potential limit to the present study. Further analysis in regards to insulin may uncover significant findings among Apolipoprotein-A1 and Apolipoprotein-B in the future. Additionally, it may highlight discrepancies among groups for weight status and/or ethnicity for insulin response to a single high fat meal.

Conclusion

Due to the relationship of impaired metabolism and metabolic diseases, as well as these ongoing health disparities, we investigated the differences in metabolic response of a single high fat meal between both lean and overweight/obese, CA, and AA women. Our findings support the primary purpose of this study to assess the metabolic differences between lean and overweight/obese women. There were notable and significant differences seen between women of different weight status, and weight status within ethnicity groups. Additionally, it supports the secondary purpose of this project to

determine effect that ethnicity plays on metabolic flexibility in response to a single high fat meal. This study revealed that there were indications of metabolic inflexibility among CA overweight/obese, AA overweight/obese and AA lean women, compared to that of CA lean women. This may help explain the attenuation of negative health implications often seen in these populations. Future research further highlighting these metabolic differences in weight status and ethnicity should be conducted to help further assess these discrepancies.

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Appendix A

IRB Stamped Informed Consents



A LEADING AMERICAN UNIVERSITY WITH INTERNATIONAL REACH
SCHOOL OF KINESIOLOGY, RECREATION & SPORT



INFORMED CONSENT DOCUMENT

Project Title: Metabolic Flexibility among Women in Response to an Acute High Fat Load

Investigators:

Alyssa Olenick - School of Kinesiology, Recreation, and Sport, (724) 504 - 8403

Dr. Jill Maples - School of Kinesiology, Recreation, and Sport, (270) 745 - 4339

You are being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your signed agreement to participate in this project.

The investigators will explain to you in detail the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask any questions you have to help you understand the project. A basic explanation of the project is written below. Please read this explanation and discuss with the researcher any questions you may have.

If you then decide to participate in the project, please sign this form in the presence of the person who explained the project to you. You should be given a copy of this form to keep.

1. Nature and Purpose of the Project:

Metabolic syndrome is often linked to obesity and is a rising concern¹. It is the combination of a variety of health conditions, such as excess body fat, high blood pressure, high cholesterol and abnormal triglyceride levels². These risk factors can lead to even more major health conditions such as cardiovascular disease, stroke, diabetes or obesity. Obesity, or excessive weight gain, it's self is also a serious public health concern as it contributes to inflammation, and insulin resistance - all of which negatively affect health. Metabolic flexibility is a person's ability to adapt to utilize the available fuel source, such as fat or carbohydrate, found in the human diet. Contrastingly, metabolic inflexibility is the inability to utilize a given fuel source, such as the switch into fat metabolism after the consumption of a high fat meal³. In addition, physical activity improves unfavorable outcomes associated with obesity. African American women have a higher predisposal to certain disease states like obesity, coronary heart disease and metabolic syndrome¹. However, the connections between physical activity, ethnicity, inflammation, and metabolic dysfunction, particularly in response to a single high-fat meal, independent of a controlled diet, (similar to that which is typically consumed in a Western diet), among Caucasian and African American women have not been studied. This study will examine the impact of ethnicity on inflammatory and metabolic responses to a high-fat meal in Caucasian and African American women.

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Original - 8/26/2016

2. Explanation of Procedures:

The study will involve one visit to either the Exercise Laboratory within in the WKU Medical Sciences Complex at the Medical Center or Smith Stadium East in the Kinesiology Department. The night before the visit, you will be given a list of instructions for consuming a healthy meal that will not disturb your metabolism. You will be asked to not eat or drink anything besides water during the 10 hours prior to the study. Upon arrival, a medical professional will take your vital signs and ask you some questions. After that, you will be asked to lay comfortably on a bed. A clear hood-like device will be placed over your head to measure your metabolism for ~40minutes (total throughout the study session). You will be asked to sit quietly and relax during this measurement. After this measurement is complete, the nurse practitioner will do a blood draw by inserting a catheter (soft, plastic tube) in the bend of your arm or your hand. Following this blood draw, you will be provided with a high-fat meal consisting of a pre made smoothie from Smoothie King Inc. Following the ingestion of the meal, you will be asked to remain resting in the lab for four hours. During these four hours, you will be able to work, read, watch TV, use your phone, etc. Blood will be drawn three times over the course of the four hours by the nurse from the same catheter (i.e. you will only be “stuck” one time). During the last half hour, the hood-like device will be used again to measure metabolism (for approximately 10 minutes). At the end of the study visit, the catheter will be removed. After this visit, you will be given a wristwatch that monitors your physical activity levels. You will wear this watch for a week, and then return it to the lab.

3. Discomfort and Risks:

What are the risks to you?

Likely/Common

Mild

- Pain from the blood draws
- Gastrointestinal discomfort from the high-fat meal

Less Likely/Less Common

Serious

- Bruising or fainting from the blood draws
- Anxiety from the hood measurement if claustrophobic

Rare

Serious

- Infection from blood draws

4. Benefits:

You will not benefit directly from being in the study. However, you will learn information about your metabolism and other baseline health measures (e.g. body fat %, resting blood pressure, etc.). We hope the knowledge we gain will help us understand factors that influence the overall metabolic health of women. You will be paid \$50 for your participation in this study. This sum will be paid upon return of the watch used in this study.

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5. Confidentiality:

To help protect your confidentiality, we will do everything we can to keep your information private and protected. Your research file will contain identifiable information such as your name, patient ID#, and birthday. Protected Health Information (PHI) will be created by the study. Study PHI will be kept in your research record and only the research team will have access to the information. The data obtained from this study will be kept confidential. Patients are assigned a study specific identifying number (PID) upon entry to the study, after which all medical information is referenced by this number. Databases that contain private health, medical or research information are behind firewalls, require password/username for access, are maintained using the PID, and only the PI and Co-PIs, have access to the code that matches the PID with other patient identifiers. All hardcopy data records are stored in locked file cabinets and kept in a locked office. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be identified.

6. Refusal/Withdrawal:

Refusal to participate in this study will have no effect on any future services you may be entitled to from the University or from your physician. Anyone who agrees to participate in this study is free to withdraw from the study at any time with no penalty.

You understand also that it is not possible to identify all potential risks in an experimental procedure, and you believe that reasonable safeguards have been taken to minimize both the known and potential but unknown risks. If a medical emergency does occur, you understand that you are responsible for any costs incurred, including but not limited to the services of Emergency Medical Technicians, emergency room care, hospitalization, etc. We strongly encourage you to ensure that you have adequate health insurance coverage or other means of satisfying any costs for which you will be liable.

Signature of Participant

Date

Witness

Date

THE DATED APPROVAL ON THIS CONSENT FORM INDICATES THAT
THIS PROJECT HAS BEEN REVIEWED AND APPROVED BY
THE WESTERN KENTUCKY UNIVERSITY INSTITUTIONAL REVIEW BOARD
Paul Mooney, Human Protections Administrator
TELEPHONE: (270) 745-2129



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WESTERN KENTUCKY UNIVERSITY
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**INFORMED CONSENT DOCUMENT
Participant Visit #2**

Project Title: Metabolic Flexibility among Women in Response to an Acute High Fat Load
Principal Investigator: Jill Maples Ph.D., School of Kinesiology, Recreation, and Sport,
Jill.Maples@wku.edu 270-745-4339

You are being asked to participate in a project conducted through Western Kentucky University. The University requires that you give your signed agreement to participate in this project.
You must be 18 years old or older to participate in this research study.

The investigator will explain to you in detail the purpose of the project, the procedures to be used, and the potential benefits and possible risks of participation. You may ask any questions you have to help you understand the project. A basic explanation of the project is written below. Please read this explanation and discuss with the researcher any questions you may have.

If you then decide to participate in the project, please sign this form in the presence of the person who explained the project to you. You should be given a copy of this form to keep.

Nature and Purpose of the Project: The purpose of this research is to determine if there is a relationship between metabolic flexibility and fitness level among women.

To minimize risks when performing exercise interventions, you will be asked to complete some brief screening documents to ensure that you are classified as *low risk* according to the American College of Sports Medicine (ACSM). If you are classified as *moderate or high risk* according to ACSM, then you will be excluded from participation in this research study. Additionally, if you have a significant medical history, have any physical conditions that would contraindicate physical exercise, are pregnant, you will also be excluded from participating in this research.
Below is a brief description of each of the testing procedures. The investigators prior to you performing each of the procedures will provide detailed instructions. All procedures will take place in the Exercise Physiology Lab.

For your visit, you will complete a graded exercise test on a treadmill. Before the test, you will receive a finger stick to check your blood lactate and sugar levels. If you opt to receive the blood draw, you will also have blood draw taken. The blood will be drawn by putting a needle into a vein in your arm. One tube of blood will be taken. This will take about five minutes. This will be repeated again after the graded exercise test is complete. All blood draws will be performed a trained research team member. You will also be asked to take one survey about your mood before the exercise test and then two surveys about your mood and exercise enjoyment after the exercise test.

A maximal graded exercise test:

For this test, you will be given a 3-minute warm-up on a treadmill. After your warm-up, we will slowly

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increase the incline and the speed of the treadmill until you report that you are too fatigued to continue. This test will take between 8 and 12 minutes. During this test, you will have a mouth piece and nose clip on so we can monitor air consumption. Before and after the exercise test your finger will be stuck to assess blood glucose and lactate. You will be monitored very closely by a research team member during this test to assess your well being.

You will be offered a snack and monitored closely before you leave. At 6 minutes post- and 15 minutes post-exercise, we will perform a finger stick to check blood lactate and sugar levels to make sure they are at safe levels before you leave the laboratory.

Discomfort and Risks: Testing of maximal aerobic capacity can cause musculoskeletal injury, which includes bruising, muscle cramps or soreness, muscle strains and sprains or muscle tears. Risks will be reduced by demonstration of proper technique, allowing participants to practice the technique, and by allowing participants to warm up prior to all protocols.

The metabolic analyzer must be worn with a mouth piece and nose clip which could pose minimal discomfort to the participant.

Participants will be monitored during and after testing, and testing will be terminated if participants exhibit adverse signs/symptoms such as the onset of chest pains, lightheadedness, confusion, pallor, nausea, or cold, clammy skin, or if the subject feels for any other reason they need/want to stop (ACSM, 2010). In case of accident or illness, a CPR certified individual, until emergency medical services personnel arrive, would give proper care.

3. **Discomfort and Risks:**

Likely/Common

Mild

- Feeling light-headed or fatigued during or after the exercise sessions.
- Muscle soreness of the legs during or after exercise
- Discomfort from finger sticks
- If consent to blood draw, pain from needle stick

Less Likely/Less Common

Serious

- Musculoskeletal injury as a result of the exercise testing (ex: strained muscle)
- If consent to blood draws, bruising or fainting

Rare

Life Threatening

- Life threatening arrhythmia which may lead to a heart attack, and possibly death.
- If consent to blood draw, infection

4. **Benefits:**

You will learn valuable information about your fitness levels and other vitals (resting heart rate, blood pressure, etc). This study will help us learn more about the factors that influence the metabolic health of women. You will be paid \$30 for participation in the study.



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5. Confidentiality:

To help protect your confidentiality, we will do everything we can to keep your information private and protected. Your research file will contain identifiable information such as your name, patient ID#, and birthday. Protected Health Information (PHI) will be created by the study. Study PHI will be kept in your research record and only the research team will have access to the information. Patients are assigned a study specific identifying number (PID) upon entry to the study, after which all information is referenced by this number. All records generated from this research will be kept securely, and only available to the research team. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be identified.

6. Refusal/Withdrawal:

Refusal to participate in this study will have no effect on any future services you may be entitled to from the University. Anyone who agrees to participate in this study is free to withdraw from the study at any time with no penalty.

You understand also that it is not possible to identify all potential risks in an experimental procedure, and you believe that reasonable safeguards have been taken to minimize both the known and potential but unknown risks. If a medical emergency does occur, you understand that you are responsible for any costs incurred, including but not limited to the services of Emergency Medical Technicians, emergency room care, hospitalization, etc. We strongly encourage you to ensure that you have adequate health insurance coverage or other means of satisfying any costs for which you will be liable.

Signature of Participant

Date

Witness

Date

- Do we have permission to take photos during your study visits for educational purposes?

(Initial here) _____

- Do we have permission to take a blood sample before and after the exercise test?

(Initial here) _____

- My blood may be stored and used for future research on this topic by our study team.

(Initial here) _____

THE DATED APPROVAL ON THIS CONSENT FORM INDICATES THAT
THIS PROJECT HAS BEEN REVIEWED AND APPROVED BY
THE WESTERN KENTUCKY UNIVERSITY INSTITUTIONAL REVIEW BOARD
Paul Mooney, Human Protections Administrator
TELEPHONE: (270) 745-2129



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Appendix B
Health History Questionnaire

Medical History Questionnaire

MEDICAL HISTORY

Now I am going to ask you a few questions to determine if your health status

...	YES	NO
1. Any chronic illness or condition?	_____	_____
2. Recent surgery? (Last 12 months)	_____	_____
3. Pregnancy? (Now or within the last 3 months)	_____	_____
4. Are you currently or were you recently nursing?	_____	_____
5. History of breathing or lung problems?	_____	_____
6. Diabetes or thyroid conditions?	_____	_____
7. Cigarette smoking habit?	_____	_____
8. Increased blood cholesterol?	_____	_____
9. Are you aware of being allergic to any drugs or insect bites?	_____	_____
10. Do you have asthma?	_____	_____
11. Do you have epilepsy, convulsions, or seizures of any kind?	_____	_____
12. Do you follow any specific diet?	_____	_____

Please explain in detail any "YES" answers:

Date of last menstrual cycle:

Family History: Has any member of you family had any of those listed above?

Medication

Are you currently taking:

	YES	NO
1. Cholesterol-lowering medications?	_____	_____
2. Birth control (of any type)	_____	_____
3. Psychiatric medication that may alter weight?	_____	_____
4. Appetite suppressants?	_____	_____
5. Contraceptive or hormone replacement medications?	_____	_____
6. Birth control?	_____	_____
7. Blood thinning medication (NSAIDs)?	_____	_____

Please list any medications you are currently taking:

Weight status:

1. How long have you been at your current weight?
2. Have you in the past lost (or gained) any significant amount of weight?

If so, how much?

Appendix C

Pretest Meals

Lean (800 calories) Over weight (1000 calories) and obese (1200 calories) women

Pretesting Menu Guideline for Study Participants needing **800 Calories** and evening snack:

In order to prepare for the testing on the WKU, we would like to ask for you to follow a specific diet for the dinner the night before you come into the Lab.

Begin your dinner meal around 6 pm, have an evening snack around 9 pm, and then only drink water after that.

Eat all of the food on the menu to get adequate amounts of food.

Avoid alcohol for 24 hours before coming in for the testing.

Prepare the meals as stated without adding anything extra other than herbs or spices, including salt and pepper

Do not consume any caffeine or chocolate

Dinner:

3 ounces (about the size of a deck of cards) of baked, broiled or grilled chicken, beef, pork, fish or tofu

1 medium baked potato, sweet potato or equal amount of mashed potatoes, cooked pasta or rice

1 ounce sour cream or ¼ cup of gravy or sauce (like Alfredo) for potato, pasta or rice

½ cup carrots, green beans, asparagus, or other non-starchy vegetable

1 dinner roll, slice of bread or equal amount of pita, naan or corn bread

2 teaspoons margarine or butter

½ cup grapes or one small peach, orange, apple or other desired fruit

½ cup ice cream, sherbet or frozen yogurt **or** 1 serving vanilla wafers or shortbread cookies

½ cup apple or other fruit juice

Evening Snack- to be eaten at 9 pm:

3 graham cracker squares

1 cup 2% milk

Or

½ peanut butter and jelly sandwich

After that, do not eat or drink anything, except water, until after the health professional tells you may eat at the Exercise Laboratory.

Pretesting Menu Guideline for Pregnancy Study participants needing **1000 Calories** and evening snack:

In order to prepare for the testing at the Medical Center, we would like to ask for you to follow a specific diet for the dinner the night before you come to visit us.

Begin your dinner meal around 6 pm, have an evening snack around 9 pm, and then only drink water after that.

Eat all of the food on the menu to get adequate amounts of food.

Avoid alcohol for 24 hours before coming in for the testing.

Prepare the meals as stated without adding anything extra other than herbs or spices, including salt and pepper

Do not consume any caffeine or chocolate

Dinner:

3 ounces (about the size of a deck of cards) of baked, broiled or grilled chicken, beef, pork, fish or tofu

1 baked potato, sweet potato or equal amount of mashed potatoes, cooked pasta or rice

1.5 ounce sour cream or ¼ cup of gravy or sauce (like Alfredo) for potato, pasta or rice

¾ cup carrots, green beans, asparagus, or other non-starchy vegetable

1.5 dinner roll, slice of bread or equal amount of pita, naan or corn bread

1.5 teaspoons margarine or butter

¾ cup grapes or one small peach, orange, apple or other desired fruit

½ cup ice cream, sherbet or frozen yogurt **or** 1 serving vanilla wafers or shortbread cookies

¾ cup apple or other fruit juice

Evening Snack- to be eaten at 9 pm:

3 graham cracker squares

1 cup 2% milk

Or

½ peanut butter and jelly sandwich

After that, do not eat or drink anything, except water, until after your nurse tells you may eat at the Exercise Laboratory.

Pretesting Menu Guideline for Research Participants needing **1200 Calories** and evening snack:

In order to prepare for the testing at WKU, we would like to ask for you to follow a specific diet for the dinner the night before you come into the Lab.

Begin your dinner meal around 6 pm, eat your evening snack around 9 pm, and then drink water only after that.

Eat all of the food on the menu to get adequate amounts of food.

Avoid alcohol for 24 hours before coming in for the testing.

Prepare the meals as stated without adding anything extra other than herbs or spices, including salt and pepper

Do not consume any caffeine or chocolate

Dinner- to be eaten around 6 pm:

3 ounces (about the size of a deck of cards) of baked, broiled or grilled chicken, turkey, beef, pork, fish or tofu

1 large baked potato, sweet potato or equal amount of mashed potatoes, cooked pasta or rice

2 ounces sour cream or ½ cup of gravy or sauce (like Alfredo) for potato, pasta or rice

1 cup carrots, green beans, asparagus, or other non-starchy vegetable

2 dinner rolls, 2 slices of bread or equal amounts of pita, naan or corn bread

1 tablespoon margarine or butter

1 cup grapes or one large peach, orange, apple or other desired fruit

1 slice cheese or small carton of yogurt

½ cup ice cream, sherbet or frozen yogurt **or** 1 serving vanilla wafers or shortbread cookies

1 cup of skim or low fat milk

Evening Snack- to be eaten at 9 pm:

3 graham cracker squares

1 cup 2% milk

Or

½ peanut butter and jelly sandwich

After that, do not eat or drink anything, except water, until after your nurse tells you may eat at the Exercise Laboratory.

Appendix D

International Physical Activity Questionnaire (IPAQ)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

☐ Yes

☐ No



Skip to PART 2: TRANSPORTATION

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

_____ days per week

☐

No vigorous job-related physical activity



Skip to question 4

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

_____ hours per day

_____ minutes per day

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

_____ days per week

☐

No moderate job-related physical activity



Skip to question 6

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5. How much time did you usually spend on one of those days doing moderate physical activities as part of your work?
- _____ hours per day
_____ minutes per day
6. During the last 7 days, on how many days did you walk for at least 10 minutes at a time as part of your work? Please do not count any walking you did to travel to or from work.
- _____ days per week
- ☐ No job-related walking → Skip to PART 2: TRANSPORTATION
7. How much time did you usually spend on one of those days walking as part of your work?
- _____ hours per day
_____ minutes per day

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the last 7 days, on how many days did you travel in a motor vehicle like a train, bus, car, or tram?
- _____ days per week
- ☐ No traveling in a motor vehicle → Skip to question 10
9. How much time did you usually spend on one of those days traveling in a train, bus, car, tram, or other kind of motor vehicle?
- _____ hours per day
_____ minutes per day

Now think only about the bicycling and walking you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the last 7 days, on how many days did you bicycle for at least 10 minutes at a time to go from place to place?
- _____ days per week
- ☐ No bicycling from place to place → Skip to question 12

11. How much time did you usually spend on one of those days to bicycle from place to place?
- _____ hours per day
_____ minutes per day
12. During the last 7 days, on how many days did you walk for at least 10 minutes at a time to go from place to place?
- _____ days per week
- ☐ No walking from place to place → **Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY**
13. How much time did you usually spend on one of those days walking from place to place?
- _____ hours per day
_____ minutes per day

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the last 7 days in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, chopping wood, shoveling snow, or digging in the garden or yard?
- _____ days per week
- ☐ No vigorous activity in garden or yard → **Skip to question 16**
15. How much time did you usually spend on one of those days doing vigorous physical activities in the garden or yard?
- _____ hours per day
_____ minutes per day
16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, sweeping, washing windows, and raking in the garden or yard?
- _____ days per week
- ☐ No moderate activity in garden or yard → **Skip to question 18**

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17. How much time did you usually spend on one of those days doing moderate physical activities in the garden or yard?

_____ hours per day
_____ minutes per day

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate activities like carrying light loads, washing windows, scrubbing floors and sweeping inside your home?

_____ days per week

☐

No moderate activity inside home



**Skip to PART 4: RECREATION,
SPORT AND LEISURE-TIME
PHYSICAL ACTIVITY**

19. How much time did you usually spend on one of those days doing moderate physical activities inside your home?

_____ hours per day
_____ minutes per day

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the last 7 days solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the last 7 days, on how many days did you walk for at least 10 minutes at a time in your leisure time?

_____ days per week

☐

No walking in leisure time



Skip to question 22

21. How much time did you usually spend on one of those days walking in your leisure time?

_____ hours per day
_____ minutes per day

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do vigorous physical activities like aerobics, running, fast bicycling, or fast swimming in your leisure time?

_____ days per week

☐

No vigorous activity in leisure time



Skip to question 24